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CERTIFICATION REGARDING SEQUENCE LISTING AND PRELIMINARY AMENDMENT	
Address to: Mail Stop Missing Parts Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	
Attorney Docket	STAN-303
First Named Inventor	MONJE, MICHELLE L.
Application Number	10/826,472
Filing Date	April 16, 2004
Confirmation Number	1490
Group Art Unit	1636
Examiner Name	
Title: "PREVENTION OF DEFICITS IN NEUROGENESIS WITH ANTI-INFLAMMATORY AGENTS"	

Sir:

Prior to the examination of the above-referenced application on the merits, please enter the amendments below.

AMENDMENTS

In the Specification:

Please replace paragraph 101 in its entirety with the following:

[101] Total RNA isolation, cDNA synthesis, and SYBR Green real-time quantitative RT-PCR. Total RNA was isolated from neural precursor cell cultures using RNeasy mini kit (Qiagen) and synthesis of cDNA was performed using the SuperScript First-strand Synthesis System for RT-PCR (Invitrogen). Quantitative SYBR Green real time PCR was carried out as described previously. Briefly, each 25 µl SYBR green reaction consisted of 5 µl of cDNA (50 ng/µl), 12.5 µl of 2x Universal SYBR Green PCR Master Mix (PerkinElmer Life Sciences) and 3.75 µl of 50 nM forward and reverse primers. Optimization was performed for each gene-specific primer prior to the experiment to confirm that 50 nM primer concentrations did not produce nonspecific primer-dimer amplification signal in no-template control tubes. Primer sequences were designed using Primer Express Software. Quantitative RT-PCR was performed on ABI 5700 PCR instrument (PerkinElmer Life Sciences) by using 3-stage program parameters provided by the manufacturer as follows; 2 min at 50°C, 10 min at 95°C, and then 40 cycles of 15 s at 95°C and 1 min at 60 °C. Specificity of the produced amplification product was confirmed by examination of dissociation reaction plots. A distinct single peak indicated that single DNA sequence was amplified during PCR. In addition, end

reaction products were visualized on ethidium bromide-stained 1.4% agarose gels. Appearance of a single band of the correct molecular size confirmed specificity of the PCR. Each sample was tested in five copies with quantitative PCR, and samples obtained from three independent experiments were used to calculate the means and standard deviations. Primers were as follows (F=forward, R=reverse):

GAPDH F	AAGAGAGAGGCCCTCAGTTGCT	SEQ. ID 1
GAPDH R	TTGTGAGGGAGATGCTCAGTGT	SEQ. ID 2
MASH1 F	GACAGGCCCTACTGGGAATG	SEQ. ID 3
MASH1 R	CGTTGTCAAGAAACACTGAAGACA	SEQ. ID 4
HES1 F	CGGCTTCAGCGAGTGCAT	SEQ. ID 5
HES1 R	CGGTGTTAACGCCCTCACA	SEQ. ID 6
HES5 F	GGAGGCGGTGCAGTTCCT	SEQ. ID 7
HES5 R	GGAGTGGTAAAGCAGCTTCATC	SEQ. ID 8
NEUROD F	GGACAGACGAGTGCCTCAGTTC	SEQ. ID 9
NEUROD R	TCATGGCTTCAAGCTCATCCTCCT	SEQ. ID 10

Please insert the attached "Sequence Listing" as separately numbered pages 1 - 3 after the abstract.